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Microbiological Evaluation of Garlic Extract and SwissADME Profiling of Allicin as an Antimicrobial Candidate

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ABSTRACT

This study aimed to evaluate the microbiological quality of garlic extract using a rapid ready-to-use plate system (Easy Plate) and to assess the physicochemical and pharmacokinetic properties of allicin through in silico analysis using SwissADME. Microbiological testing was performed on garlic extract samples targeting coliforms, *Staphylococcus aureus*, and Enterobacteriaceae. The in silico analysis evaluated physicochemical characteristics, solubility, pharmacokinetic properties, and drug-likeness parameters of allicin. The microbiological results showed no detectable bacterial growth in the tested garlic extract samples under the applied conditions. SwissADME analysis indicated that allicin has favorable properties, including compliance with Lipinski's Rule of Five, balanced lipophilicity, good predicted water solubility, and high gastrointestinal absorption. These findings suggest that garlic extract exhibited acceptable microbiological quality under the present test conditions, while allicin demonstrated promising drug-like characteristics as a bioactive organosulfur compound. However, the microbiological findings should be interpreted cautiously because the intrinsic antimicrobial activity of garlic extract may affect microbial recovery, and the in silico results remain predictive and require further experimental validation.

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ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi kualitas mikrobiologi ekstrak bawang putih menggunakan sistem rapid ready-to-use plate (Easy Plate) serta menilai sifat fisikokimia dan farmakokinetik allicin melalui analisis *in silico* menggunakan SwissADME. Pengujian mikrobiologi dilakukan pada sampel ekstrak bawang putih dengan target coliform, *Staphylococcus aureus*, dan Enterobacteriaceae. Analisis *in silico* digunakan untuk mengevaluasi karakteristik fisikokimia, kelarutan, sifat farmakokinetik, dan parameter *drug-likeness* dari allicin. Hasil uji mikrobiologi menunjukkan bahwa tidak ditemukan pertumbuhan bakteri yang terdeteksi pada sampel ekstrak bawang putih dalam kondisi pengujian yang digunakan. Analisis SwissADME menunjukkan bahwa allicin memiliki karakteristik yang menguntungkan, termasuk memenuhi Lipinski's Rule of Five, memiliki lipofilisitas yang seimbang, kelarutan air terprediksi yang baik, dan absorpsi gastrointestinal yang tinggi. Temuan ini menunjukkan bahwa ekstrak bawang putih memiliki kualitas mikrobiologi yang dapat diterima pada kondisi pengujian saat ini, sementara allicin menunjukkan karakteristik *drug-like* yang menjanjikan sebagai senyawa organosulfur bioaktif. Namun, hasil mikrobiologi perlu ditafsirkan secara hati-hati karena aktivitas antimikroba intrinsik ekstrak bawang putih dapat memengaruhi pemulihan mikroba, dan hasil *in silico* tetap bersifat prediktif sehingga memerlukan validasi eksperimental lebih lanjut.

Kata Kunci: Ekstrak bawang putih; Allicin; Kualitas mikrobiologi; Easy Plate; SwissADME

1. Introduction

Garlic (*Allium sativum*) is widely recognized as both a culinary ingredient and a medicinal plant because of its rich content of organosulfur compounds, particularly allicin, which have been associated with significant antimicrobial activity. Consequently, garlic extract has been increasingly developed as a functional food ingredient and nutraceutical product. However, like other processed natural products, garlic extract remains vulnerable to microbial contamination, which may compromise product safety, stability, and overall quality [1].

Ensuring microbiological quality is therefore essential, especially for products intended for human consumption. Microbiological evaluation commonly involves the detection of indicator microorganisms that reflect hygienic quality and possible contamination during processing. Among the most relevant microbial groups for this purpose are coliforms, *Staphylococcus aureus*, and Enterobacteriaceae. Conventional microbiological methods, such as pour plate and spread plate techniques, are generally reliable, but they have important limitations, including long incubation times and reduced operational efficiency, which may delay quality-control decisions [2].

To overcome these limitations, rapid microbiological methods such as ready-to-use plate systems (Easy Plate Systems) have been introduced. These systems offer practical advantages, including simplified handling, reduced analysis time, and better procedural standardization. Nevertheless, their application to complex matrices such as garlic extract remains insufficiently explored. Garlic extract contains intrinsic antimicrobial compounds, especially allicin, which may suppress microbial growth during incubation and influence colony recovery. As a result, the use of rapid plate-based methods in antimicrobial-rich natural products requires careful evaluation to determine whether the observed microbiological findings truly reflect product quality or are partly affected by matrix-related inhibition [3].

In addition to its relevance in product safety, allicin has been extensively studied as a bioactive organosulfur compound with antimicrobial potential. Its activity is primarily associated with its interaction with thiol-containing enzymes and proteins in microbial cells, leading to disruption of essential cellular functions. Evaluating the physicochemical and pharmacokinetic properties of allicin is therefore important for understanding its potential as an antimicrobial candidate. In this context, *in silico* tools such as SwissADME provide a rapid and efficient approach for predicting drug-likeness and ADME-related properties. However, these computational evaluations are often discussed separately from experimental microbiological assessments, resulting in limited integration between compound-level prediction and product-level microbial evaluation [4],[5].

The primary focus of this study was the microbiological evaluation of garlic extract using a rapid ready-to-use plate system (Easy Plate). In addition, this study incorporated SwissADME-based profiling of allicin as a supporting component to provide complementary insight into the antimicrobial relevance of the major organosulfur compound present in garlic. The novelty of this study lies in the integration of descriptive microbiological quality assessment of garlic extract with computational profiling of allicin, thereby providing a broader perspective that links product quality with the predicted functional characteristics of its bioactive constituent.

2. Methods

Study Design

This study used a mixed experimental-computational design consisting of two complementary components. The experimental component involved descriptive microbiological evaluation of garlic extract using a rapid ready-to-use plate system (Easy Plate), while the computational component involved *in silico* profiling of allicin using the SwissADME platform. The microbiological evaluation was conducted to assess the presence of selected microbial indicators in garlic extract under the applied test conditions, whereas the SwissADME analysis was performed to provide supporting information on the physicochemical and pharmacokinetic properties of allicin as the major bioactive organosulfur compound in garlic [6],[7].

Sample Preparation

The sample used in this study was ethanol extract of garlic (*Allium sativum*). Prior to microbiological testing, the extract was diluted in sterile Buffered Peptone Water (BPW) to minimize possible interference from residual solvent and to provide a more suitable medium for microbial recovery. A total of 1 g of garlic extract was diluted in 100 mL of sterile BPW and homogenized before analysis [1],[8].

Microbiological Analysis

Microbiological analysis was performed directly on the garlic extract samples to evaluate three target microbial groups, namely coliforms, *Staphylococcus aureus*, and Enterobacteriaceae. The analysis was conducted descriptively to determine whether detectable bacterial contamination was present in the tested samples under the applied conditions. No artificially inoculated samples or recovery controls were included in the present study. All microbiological tests were performed in triplicate to improve the consistency of observation.

The microbiological examination used commercial Easy Plate systems specific to each microbial group. For each test, 1 mL of the appropriately diluted sample was aseptically pipetted onto the center of the Easy Plate and allowed to spread through capillary action according to the manufacturer's instructions. The plates were then

incubated under the recommended conditions: coliforms at 35 °C for 24 h, *Staphylococcus aureus* at 35 °C for 24–48 h, and Enterobacteriaceae at 35 °C for 24 h. Colony growth was interpreted based on the color indicators provided by the Easy Plate system, and the results were recorded as detectable or non-detectable bacterial growth, with microbial findings expressed descriptively in CFU/g where applicable [9].

SwissADME *In Silico* Analysis

The *in silico* analysis of allicin was carried out using the SwissADME web server. The canonical SMILES structure of allicin was obtained from the PubChem database and entered into the SwissADME platform for analysis. The evaluated parameters included physicochemical properties, water solubility, lipophilicity, pharmacokinetic characteristics, and drug-likeness based on Lipinski's Rule of Five. The output was interpreted descriptively to assess the predicted suitability of allicin as a bioactive compound with potential antimicrobial relevance [7],[10].

Statistical Analysis

Because the microbiological component of this study was descriptive and the *in silico* component generated predictive computational outputs, the data were analyzed descriptively. Microbiological observations from triplicate tests were summarized based on the presence or absence of detectable growth and reported in relation to the tested microbial groups. SwissADME results were interpreted based on the output parameters provided by the platform, without inferential statistical testing.

3. Results and Discussion

Physicochemical Properties and Drug-Likeness of Allicin

The physicochemical properties and drug-likeness profile of allicin predicted by SwissADME are presented in **Table 1** and **Table 2**. In the present analysis, allicin was identified by the molecular formula C8H10OS2, molecular weight 182.27 g/mol, and canonical SMILES C=CCSS(=O)CC=C. The compound showed favorable aqueous solubility across multiple prediction models, ranging from soluble to very soluble. In addition, allicin had one hydrogen-bond acceptor, no hydrogen-bond donor, and a topological polar surface area (TPSA) of 61.58 Å², suggesting a polarity range compatible with membrane permeation. These parameters indicate that allicin possesses a physicochemical profile consistent with the requirements generally associated with orally active small molecules [7],[10],[13].

Table 1. Physicochemical Properties and Solubility Profile of Allicin Predicted by SwissADME

Parameter	Value	Interpretation
Compound name	Allicin	Organosulfur compound analyzed in SwissADME
Molecular formula	C8H10OS2	Chemical identity of the analyzed molecule
Molecular weight	182.27 g/mol	Within the acceptable range for small-molecule drug candidates
SMILES	C=CCSS(=O)CC=C	Canonical structure used for SwissADME input
Log S (ESOL)	-1.34	Very soluble
Log S (Ali)	-2.20	Soluble
Log S (SILICOS-IT)	-1.70	Soluble
H-bond acceptors	1	Low

H-bond donors	0	Very low
TPSA	61.58 Å²	Supports potential membrane permeability

In terms of lipophilicity, allicin had a consensus Log P value of 1.81, which falls within the commonly acceptable range for orally absorbed compounds. This value indicates balanced lipophilicity, meaning that the compound is neither excessively hydrophilic nor excessively lipophilic. Drug-likeness assessment based on Lipinski's Rule of Five further showed that allicin met all major criteria without any violations. Its molecular weight, Log P value, hydrogen-bond donor value, and hydrogen-bond acceptor value were all within the acceptable limits. Collectively, these findings suggest that allicin has a computationally favorable physicochemical and drug-like profile. However, because these results are derived from in silico prediction, they should be interpreted as supportive rather than definitive evidence of pharmacological suitability [7],[10],[13].

Table 2. Lipophilicity and Drug-Likeness Profile of Allicin Based on SwissADME

A. Lipophilicity

Parameter	Value	Interpretation
Consensus Log P o/w	1.81	Balanced lipophilicity
Log P range (WLOGP, XLOGP3, etc.)	1.18–2.82	Within the expected range for oral compounds

B. Lipinski's Rule of Five

Rule	Limit (Max)	Observed Value	Violation
Molecular weight	500	182.27	0
Log P o/w	5	1.81	0
H-bond donors	5	0	0
H-bond acceptors	10	1	0

Note: Allicin showed 0 violations of Lipinski's Rule of Five.

Pharmacokinetic Profile Based on SwissADME Prediction

The pharmacokinetic profile of allicin predicted by SwissADME is presented in **Table 3**. The analysis indicated that allicin has high gastrointestinal absorption, suggesting a favorable probability for oral uptake. In addition, allicin was predicted to be non-substrate of P-glycoprotein (P-gp), which may support intestinal absorption by reducing the likelihood of active efflux through this transporter. These findings suggest that allicin has a potentially favorable absorption profile based on computational prediction [7],[14].

SwissADME also predicted that allicin is BBB permeant, indicating a theoretical capacity to cross the blood–brain barrier. The predicted log Kp value of -8.36 cm/s reflects very low skin permeability, which is consistent with a compound that is not primarily optimized for transdermal penetration. In addition, allicin was predicted not to inhibit the major CYP450 isoenzymes evaluated, including CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. This profile suggests a potentially low risk of CYP-mediated drug–drug interactions, at least from the perspective of computational screening [7],[14],[15].

Overall, the SwissADME output suggests that allicin has a computationally favorable pharmacokinetic profile, particularly with respect to gastrointestinal absorption and the absence of major CYP inhibition. However, these results should be interpreted with caution because SwissADME provides predictive in silico estimations rather than direct experimental evidence. In the case of allicin, this point is especially

important because the compound is known to be chemically reactive and unstable, meaning that actual biological behavior may differ from theoretical pharmacokinetic predictions. Therefore, the present findings should be regarded as supportive preliminary information rather than definitive confirmation of in vivo pharmacokinetic performance [7],[14],[15].

Table 3. Pharmacokinetic Profile of Allicin Predicted by SwissADME

Parameter	Predicted Result	Interpretation
GI absorption	High	Suggests favorable oral absorption potential
BBB permeant	Yes	Indicates possible blood–brain barrier penetration
P-gp substrate	No	Suggests the compound is not actively effluxed by P-gp
Log Kp (skin permeation)	-8.36 cm/s	Indicates very low skin permeability
CYP1A2 inhibitor	No	Low predicted risk of CYP1A2-mediated interaction
CYP2C19 inhibitor	No	Low predicted risk of CYP2C19-mediated interaction
CYP2C9 inhibitor	No	Low predicted risk of CYP2C9-mediated interaction
CYP2D6 inhibitor	No	Low predicted risk of CYP2D6-mediated interaction
CYP3A4 inhibitor	No	Low predicted risk of CYP3A4-mediated interaction

Microbiological Findings of Garlic Extract Using Easy Plate Systems

The microbiological findings of garlic extract obtained using Easy Plate systems are summarized in **Table 4**, while representative plate observations are shown in **Figure 1**. In the present study, the microbiological evaluation targeted three indicator groups, namely coliforms, *Staphylococcus aureus*, and Enterobacteriaceae. Under the applied test conditions, no detectable bacterial growth was observed in any of the tested groups. These findings suggest that the garlic extract sample exhibited acceptable microbiological quality with respect to the selected microbial indicators.

The absence of detectable growth should, however, be interpreted cautiously. Garlic extract contains intrinsic antimicrobial compounds, particularly organosulfur constituents such as allicin, which may suppress microbial recovery during incubation. Therefore, the observed negative findings may reflect either genuinely low microbial contamination or inhibition of microbial growth by the sample matrix. Because the present study did not include inoculated recovery controls or reference method comparison, the results are best interpreted as a descriptive microbiological screening outcome rather than as definitive proof of sterility or full method performance.

Table 4. Microbiological Findings of Garlic Extract Using Easy Plate Systems

Target microorganism	Test system	Result under applied conditions	Interpretation
Target microorganism	Easy Plate Coliform	No detectable growth	Not detected under test conditions
Coliforms	Easy Plate *S. aureus*	No detectable growth	Not detected under test conditions
Staphylococcus aureus	Easy Plate Enterobacteriaceae	No detectable growth	Not detected under test conditions

Note: *Results are presented descriptively based on triplicate testing under the applied Easy Plate conditions. The absence of detectable growth should be interpreted cautiously because the intrinsic antimicrobial activity of garlic extract may influence microbial recovery*

From a product-quality perspective, the microbiological observations remain relevant because they indicate that no detectable contamination by coliforms, *Staphylococcus aureus*, or Enterobacteriaceae was identified under the current Easy Plate conditions. Nevertheless, further studies incorporating spiked controls, recovery testing, and side-by-side comparison with conventional plate methods would be necessary to confirm the reliability of microbial detection in antimicrobial-rich matrices such as garlic extract.

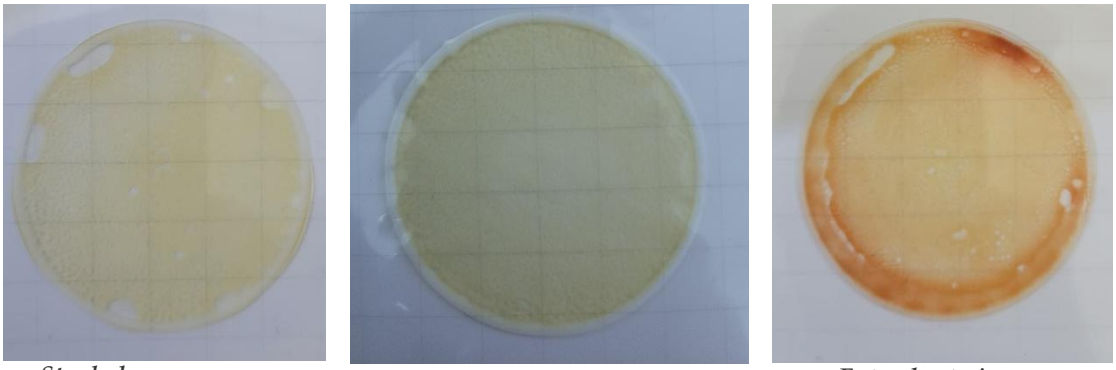

Figure 1. Representative Easy Plate results for coliforms, *Staphylococcus aureus*, and Enterobacteriaceae in garlic extract samples

Antimicrobial Relevance of Allicin: Literature-Based Interpretation

The antimicrobial relevance of allicin has been widely discussed in the literature and provides an important context for interpreting the present findings. Allicin is the principal reactive organosulfur compound formed from garlic and is recognized as one of the main contributors to the biological activity of *Allium sativum* [1],[11]. Its antimicrobial action is primarily associated with its chemical reactivity toward thiol-containing proteins and enzymes in microbial cells. Through interaction with sulfhydryl (-SH) groups, allicin may disrupt essential enzymatic functions, alter membrane-associated processes, and impair microbial survival [11],[12]. This mechanism supports the long-recognized antimicrobial relevance of garlic-derived sulfur compounds and helps explain why garlic extract may exhibit inhibitory effects against microbial growth.

Previous studies have shown that garlic-derived organosulfur compounds can act against a broad range of microorganisms, including Gram-positive bacteria such as *Staphylococcus aureus* [1],[4]. In particular, allicin has been reported to interfere with cell membrane integrity and with intracellular thiol-dependent pathways, while related

compounds such as diallyl disulfide (DADS) also exhibit antibacterial and antifungal activities [1],[11]. These observations indicate that the antimicrobial activity of garlic is not limited to a single mechanism, but may involve multiple sulfur-containing constituents with overlapping biological effects.

In the context of the present study, the absence of detectable bacterial growth in the garlic extract samples may be interpreted in light of these literature findings. However, this relationship should be treated cautiously. The present study did not directly quantify allicin concentration, isolate individual sulfur compounds, or perform controlled antimicrobial challenge testing. Therefore, the antimicrobial relevance of allicin in this manuscript should be understood as a literature-based explanation that supports, but does not prove, the microbiological observations obtained from the garlic extract matrix [1], [4], [12].

Table 5. Structurally Related Organosulfur Compounds and Their Known Antimicrobial Relevance

Related compound	Chemical structure	Relation to allicin	Reported relevance
Allicin (diallyl thiosulfinate)	C=CCSS(=O)CC=C	Principal reactive organosulfur compound in garlic	Reported as a major contributor to garlic's antimicrobial activity; reacts with thiol-containing proteins and enzymes [1],[11],[12]
Diallyl disulfide (DADS)	C=CCSSC=C	Structurally related sulfur compound and decomposition product associated with garlic chemistry	Reported to show antibacterial and antifungal activity and to contribute to the biological profile of garlic-derived sulfur compounds [1],[11]

Integrated Discussion of *In Silico* and Microbiological Findings

The integration of the microbiological findings and *in silico* analysis provides a broader interpretation of the potential relevance of garlic extract and its major organosulfur constituent, allicin. Under the applied Easy Plate conditions, no detectable growth of coliforms, *Staphylococcus aureus*, or Enterobacteriaceae was observed in the tested garlic extract samples. At the same time, SwissADME prediction indicated that allicin possesses favorable physicochemical and pharmacokinetic characteristics, including balanced lipophilicity, acceptable polarity, good predicted solubility, and high gastrointestinal absorption. Taken together, these findings suggest that garlic extract may represent a microbiologically acceptable product matrix under the present test conditions, while allicin shows a computational profile compatible with further consideration as a bioactive antimicrobial-related compound [7],[10],[13].

From a mechanistic perspective, the absence of detectable microbial growth may be interpreted in light of the known reactivity of allicin and related garlic-derived organosulfur compounds. The balanced lipophilicity of allicin predicted by SwissADME may support interaction with biological membranes, while its documented reactivity toward thiol-containing proteins provides a plausible explanation for antimicrobial action reported in the literature [1],[11],[12]. However, in the context of the present study, this interpretation must remain cautious. The microbiological findings do not directly demonstrate that allicin was responsible for the absence of detectable growth,

because the extract matrix itself may contain multiple bioactive constituents and because no recovery validation or inoculated controls were included to distinguish true absence of contamination from growth suppression during testing.

Therefore, the relationship between the Easy Plate observations and the SwissADME profile of allicin should be regarded as complementary rather than confirmatory. The microbiological data describe the outcome observed in the tested garlic extract under the applied conditions, whereas the SwissADME results provide predictive support for the potential relevance of allicin as a biologically active compound. These two components strengthen the overall rationale of the study when interpreted together, but they do not yet establish a direct causal link between allicin properties and the microbiological outcome. Further studies involving controlled inoculation experiments, microbial recovery assessment, quantitative antimicrobial testing, and direct measurement of allicin content are needed to verify whether the reduced or non-detectable microbial growth is truly associated with the antimicrobial activity of garlic-derived organosulfur compounds.

Study Limitations

This study has several limitations that should be considered in interpreting the findings. First, the microbiological analysis was conducted directly on garlic extract samples without inoculated controls, recovery validation, or comparison with conventional reference methods, which limits the ability to distinguish true absence of contamination from possible growth suppression caused by the extract matrix. Second, the microbiological evaluation was restricted to three indicator groups, namely coliforms, *Staphylococcus aureus*, and Enterobacteriaceae, and therefore does not represent a complete microbiological quality profile. Third, the Easy Plate results were interpreted descriptively under the applied test conditions, so the findings should not be regarded as full method validation. Fourth, the *in silico* analysis of allicin using SwissADME provides predictive information only and does not reflect actual biological conditions such as instability, metabolism, or degradation of the compound in real systems. Therefore, further studies incorporating inoculated recovery controls, comparative microbiological methods, quantitative antimicrobial testing, and experimental pharmacokinetic evaluation are needed to strengthen the present findings.

4. Conclusion

This study showed that garlic extract tested using the Easy Plate system exhibited no detectable growth of coliforms, *Staphylococcus aureus*, and Enterobacteriaceae under the applied test conditions, indicating acceptable microbiological quality within the scope of the present analysis. In addition, SwissADME profiling suggested that allicin possesses favorable physicochemical, drug-likeness, and predicted pharmacokinetic properties, supporting its relevance as a bioactive organosulfur compound. However, the microbiological findings should be interpreted cautiously because the intrinsic antimicrobial properties of garlic extract may influence microbial recovery, and the *in silico* results remain predictive rather than confirmatory. Therefore, further studies involving recovery validation, controlled antimicrobial testing, and experimental pharmacokinetic evaluation are required to strengthen these findings.

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Conflict of Interest:

The authors declare that there is no conflict of interest regarding the publication of this article.

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